REMARKS

Applicants acknowledge with appreciation that in the Advisory Action dated March 15, 2007, the U.S. Patent & Trademark Office (Hereafter "the Patent Office") considered and commented on the amendments and statements in the previous Response to the Final Official Action dated November 30, 2006.

I. Status Summary

Claims 12 and 24-32 are pending and have been examined by the U.S. Patent and Trademark Office (hereinafter "the Patent Office"). Claims 12 and 24-32 currently stand rejected.

Claims 12 and 24-29 currently stand rejected under 35 U.S.C. § 102(a) as being anticipated by Jonuleit *et al.* (2000) *J. Exp. Med.* 192: 1213-1222. (hereinafter "Jonuleit *et al.*").

Claims 12 and 24-30 currently stand rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,803,036 to Horwitz *et al.* (hereinafter "Horwitz *et al.*).

Claim 31 currently stands rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Horwitz *et al.*

Claim 32 currently stands rejected under under 35 U.S.C. § 103(a) as allegedly being obvious over Horwitz *et al.* in view of Jonuleit *et al.*

Claim 12 has been amended in response to the comments in the Advisory Action to clarify, as previously discussed by Applicants, that the claimed method includes the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification, including particularly at page 4, lines 25-29 (describing the embodiments of the invention) and at page 13, lines 5-7 (distinguishing the teachings of the Jonuleit reference).

Claim 25 has been amended to maintain proper antecedent basis.

No new matter has been added by way of amendment. Reexamination and reconsideration of the claims is respectfully requested.

II. The Claims Meet the Requirements of 35 U.S.C. §102

The Official Action (dated November 30, 2006, page 2) rejected claims 12 and 24-29 under 35 U.S.C. § 102(a) as being anticipated by Jonuleit *et al*. In particular, the Patent Office asserts that on page 1214 (second column, 4th paragraph, lines 1-6, and Figure 4) Jonuleit *et al*. disclose a method to identify, monitor and/or remove $CD4^+CD25^+$ cells from human blood by contacting the blood with CD4 and/or CD25 and/or CTLA-4 specific antibodies. Applicants then amended independent claim 12 to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. In the Advisory Action, the Patent Office asserted that these amendments did not overcome the rejection of record because "the claimed method uses open claim language with regard to the number of steps and thus encompasses embodiments where the blood cells may be cultured or processed (*i.e.*, stimulated) before [being] directly contacted with CD4 and CD25 antibody."

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully disagree with the Patent Office's contentions, and traverse the rejection and submit the following remarks.

In order to advance prosecution and without conceding to the Patent Office's assertions, Applicants have further amended independent claim 12 (and thus also claims 24-32, which depend from or incorporate the limitations of claim 12) to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification, including particularly at page 4, lines 25-29 (describing the embodiments of the invention) and at page 13, lines 5-7 (distinguishing the teachings of Jonuleit *et al*.). Claim 12 now further clarifies that the claimed method includes contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells and identifying, monitoring, and/or removing said $CD4^+$ $CD25^+$ regulatory T cells from the human blood, wherein no stimulation with cytokines or dendritic cells occurs between these steps.

This amendment is believed to distinguish the claimed subject matter over Jonuleit *et al*. Jonuleit *et al*. is entitled: "Induction of Interleukin 10-producing, Nonproliferating $CD4^+$ T Cells with Regulatory Properties by Repetitive Stimulation with Allogeneic Immature Human Dendritic Cells." That is, Jonuleit *et al*. teach the induction of regulatory T cells from human naïve T cells *in vitro* by repetitive stimulation with immature dendritic cells. Particularly, Jonuleit *et al*. teach the removal of naïve $CD4^+$ T cells from cord blood using CD4 MACS beads alone and the removal of naïve $CD4^+$ T cells from peripheral blood using a CD4/CD45RA Multisort kit (see Jonuleit *et al*. at page 1214, second column, 4^{th} paragraph, lines 1-6). CD45RA is not the equivalent of CD25 or CTLA-4. Jonuleit *et al*.'s Figure 4 shows that after stimulation of the T cells *in vitro* by coculturing them with allogeneic DCs for 42 hours, alloreactive T cells have a $CD25^+$ phenotype (see Figure 4 legend, page 1216). Thus, in contrast to the regulatory T cells provided by the presently claimed method, the population of cells described by Jonuleit *et al*. is not a naturally occurring population.

Indeed, Jonuleit *et al*. teaches that the naïve T cells initially isolated from blood have properties very different from those induced by stimulation with iDCs. The following passage from Jonuleit *et al*. discusses the *in vitro* generation of cells with a cytokine profile characteristic of Tr1 cells and with regulatory function (see page 1216, right column, first paragraph):

> [N]aive T cells primed and restimulated with allogeneic iDC showed a Th0 cytokine profile after the first restimulation (synthesis of intermediate amounts of IFNγ and IL-4). After repetitive stimulation with iDCs, the alloreactive T cells lost their capacity to synthesize IFN-γ, IL-2 and IL-4, indicating that these T cells did not differentiate into Th2 cells, but showed, however, an enhanced production of IL-10. Thus, the respective T cell progeny exhibited a cytokine profile characteristic of Tr1 cells, *i.e.*, synthesis of high amounts of IL-10 and no or negligible production of IFN-γ, IL-2, IL-4 or IL-5.

Thus, Jonuleit *et al*. at best teach an *in vitro* method for producing a particular cell population. Jonuleit *et al*. do not teach the isolation of $CD4^+$ $CD25^+$ regulatory T cells from human blood in the absence of stimulation, as presently claimed. In view of these differences between Jonuleit *et al*. and the claimed subject matter, Jonuleit *et al*.

do not anticipate claims 12 and 24-29. Accordingly, Applicants respectfully request that the rejection of claims 12 and 24-29 on this basis be withdrawn.

The Official Action (dated November 30, 2006, page 4) rejected claims 12 and 24-30 under 35 U.S.C. § 102(e) as being anticipated by Horwitz *et al*. Particularly, the Patent Office asserts that Horwitz *et al*. disclose a method to identify, monitor, and/or remove $CD4^+$ $CD25^+$ cells from human blood by contacting the blood with CD4 and/or CD25 and/or CTL-A4 specific antibodies. Applicants then amended independent claim 12 to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. In the Advisory Action, the Patent Office asserts that these amendments did not overcome the rejection of record because "the claimed method uses open claim language with regard to the number of steps and thus encompasses embodiments where the blood cells may be cultured or processed (*i.e.*, stimulated) before [being] directly contacted with CD4 and CD25 antibody."

After careful review of the instant rejection and the Patent Office's basis therefore, Applicants respectfully disagree with the Patent Office's contentions, and traverse the rejection and submit the following remarks.

In order to advance prosecution and without conceding to the Patent Office's assertions, Applicants have further amended independent claim 12 (and thus also claims 24-32, which depend from or incorporate the limitations of claim 12) to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification, including particularly at page 4, lines 25-29 (describing the embodiments of the invention) and at page 13, lines 5-7 (distinguishing the teachings of Jonuleit *et al*.). Claim 12 now further clarifies that the claimed method includes contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells and identifying, monitoring, and/or removing said $CD4^+$ $CD25^+$ regulatory T cells from the human blood, wherein no stimulation with cytokines or dendritic cells occurs between these steps.

This amendment is believed to distinguish the claimed subject matter over Horwitz *et al*. Horwitz *et al*. do not teach the removal of $CD4^+$ $CD25^+$ regulatory T cells from human blood by contacting the blood with CD4 and CD25 and/or CTLA-4 specific antibodies, as presently claimed. Instead, as described in the "Summary of the Invention" (col. 5, lines 59 through 65), Horwitz *et al*. teach "methods for inducing T cell tolerance in a sample of *ex vivo* peripheral blood mononuclear cells (PBMCs) comprising adding a suppressive-inducing composition to the cells. The suppressive-inducing composition can be IL-2, IL-10, TGF-β, or a mixture." Thus, Horwitz *et al*. teach the induction of regulatory T cells from human naïve T cells *in vitro* by repetitive stimulation with cytokines.

Particularly, Horwitz *et al*. teach the removal of naïve $CD4^+$ T cells from blood (col. 11, lines 5-6), after which these cells are "incubated [*i.e.*, cultured *in vitro*] with [a] suppressive-inducing composition..." (see col. 13, lines 11-14). The portions of Horwitz *et al*. cited in the previous Office Action illustrate this *in vitro* stimulation. For example, Figure 9 shows results from experiments using naïve $CD4^+$ T cells that were cultured for five days prior to the experiment and stimulated with TGF-β (see Figure 9 legend in col. 7, lines 49-65). Similarly, Figure 10A shows results from experiments using "[n]aive $CD4^+$ T cells primed with irradiated allogeneic stimulator cells ± TGF- β" (see Figure 10A legend in col. 7, line 66 through col. 8, line 3). The legend to Figure 10A makes it clear that only after this *in vitro* stimulation were cells sorted into $CD25^+$ and $CD25^-$ fractions (see col. 8, lines 1-3)[1]. Figure 7 also demonstrates results obtained with "$CD4^+$ regulatory T cells induced by TGF- β" (see col. 21, lines 25-26). Thus, Horwitz *et al*. only describe experiments in which naïve $CD4^+$ T cells were stimulated *in vitro* with cytokines to induce a regulatory phenotype.

Thus, Horwitz *et al*. at best teach an *in vitro* method for producing a particular cell population. Horwitz *et al*. do not teach the isolation of $CD4^+$ $CD25^+$ regulatory T cells from human blood in the absence of stimulation, as presently claimed. In view of these differences between Horwitz *et al*. and the claimed subject matter, Horwitz *et al*. do not

[1] The Figure 10A legend (col. 7, line 66 through col. 8, line 3) clarifies that the experiments discussed in col. 21, lines 60-67, cited in the Office Action, are the same experiments.

anticipate claims 12 and 24-30. Accordingly, Applicants respectfully request that the rejection of claims 12 and 24-30 on this basis be withdrawn.

III. The Claims Meet the Requirements of 35 U.S.C. § 103

The Official Action (page 6) rejected claim 31 under 35 U.S.C. § 103(a) as being unpatentable over Horwitz *et al*. The Patent Office contends that while "Horwitz *et al*. do not teach that the $CD4^+$ $CD25^+$ cells are activated and fixed," one of ordinary skill in the art "would have been motivated to use the fixed activated $CD4^+$ $CD25^+$ cells to test its regulatory activity because said cells may be looked at under the microscope." Respectfully, Applicants do not understand or agree with this rationale. However, in order to advance prosecution, Applicants then amended independent claim 12 to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. In the Advisory Action, the Patent Office asserted that these amendments did not overcome the rejection.

After careful review of the instant rejection and the Patent Office's basis therefore, Applicants respectfully disagree with the Patent Office's contentions, and traverse the rejection and submit the following remarks.

In order to advance prosecution and without conceding to the Patent Office's assertions, Applicants have further amended independent claim 12 (and thus also claims 24-32, which depend from or incorporate the limitations of claim 12) to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification, including particularly at page 4, lines 25-29 (describing the embodiments of the invention) and at page 13, lines 5-7 (distinguishing the teachings of Jonuleit *et al*.). Claim 12 now further clarifies that the claimed method includes contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells and identifying, monitoring, and/or removing said $CD4^+$ $CD25^+$ regulatory T cells from the human blood, wherein no stimulation with cytokines or dendritic cells occurs between these steps.

As discussed above, Horwitz *et al.* do not teach the removal of $CD4^+$ $CD25^+$ regulatory T cells from human blood by contacting the blood with CD4 and CD25 and/or CTLA-4 specific antibodies, as presently claimed. Instead, Horwitz *et al.* at best teach the *in vitro* induction of regulatory T cells from $CD4^+$ T cells by stimulation with cytokines, particularly TGF-β. Because Horwitz *et al.* do not teach the method of independent claim 12, they also do not teach the method of claim 31 in which the cells obtained from the method of claim 12 are further activated and fixed. Further, since Horwitz *et al.* do not teach the method of independent claim 12, one of skill in the art would not readily be able to modify the teachings of Horwitz *et al.* to obtain the method of claim 31.

In view of the differences between Horwitz *et al.* and the claimed invention, Horwitz *et al.* do not render claim 31 obvious. Therefore, Applicants respectfully request that the rejection of claim 31 on this basis be withdrawn.

The Official Action (page 6) rejected claim 32 under 35 U.S.C. § 103(a) as being unpatentable over Horwitz *et al.* in view of Jonuleit *et al.* The Patent Office asserts that Horwitz *et al.* did not teach "analyzing the CD4+ CD25+ cells for a cytokine profile of predominant secretion of IL-10 and only low levels of IL-2, IL-4, and IFN-γ. Jonuleit *et al.* teach enhanced production of IL-10 and low production of IL-2, IL-4 and IFN-γ is a characteristic of Tr1 cells...." The Patent Office contends that it would have been obvious to test the cytokine profile as described by Jonuleit *et al.* of the cells taught by Horwitz *et al.* "because it is a characteristic of Tr1 cells." Without conceding to the Patent Office's contentions and in order to advance prosecution, Applicants amended independent claim 12 to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. In the Advisory Action, the Patent Office asserts that these amendments did not overcome the rejection.

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully disagree with the Patent Office's contentions, and traverse the rejection and submit the following remarks.

In order to advance prosecution and without conceding to the Patent Office's assertions, claim 12 (and thus also claim 32, which incorporates the limitations of claim 12) has been further amended to clarify that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification, including particularly at page 4, lines 25-29 (describing the embodiments of the invention) and at page 13, lines 5-7 (distinguishing the teachings of Jonuleit *et al.*). Claim 12 now further clarifies that the claimed method includes contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells and identifying, monitoring, and/or removing said $CD4^+$ $CD25^+$ regulatory T cells from the human blood, wherein no stimulation with cytokines or dendritic cells occurs between these steps.

As discussed above, Horwitz *et al.* and Jonuleit *et al.* both at best teach the stimulation *in vitro* of naïve $CD4^+$ T cells. The populations described by these references are not naturally occurring populations. Neither Horwitz *et al.* nor Jonuleit *et al.* teach or suggest the claimed subject matter of a method to identify, monitor and/or remove $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood, wherein no stimulation with cytokines or dendritic cells occurs. Because these references do not teach or suggest the method of claim 12, they also do not teach or suggest the method of claim 32 in which the cells obtained from the method of claim 12 are further analyzed.

In view of the differences between the cited references and the claimed subject matter, claim 32 is not rendered obvious. Therefore, Applicants respectfully request that the rejection of claim 32 on this basis be withdrawn.

CONCLUSION

In view of the above amendments and remarks, Applicants respectfully submit that all rejections have been overcome and that the claims are in condition for allowance and an early notice to such effect is earnestly solicited.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

DEPOSIT ACCOUNT

No additional fees or extensions of time are believed to be due in connection with this communication except for those indicated in documents accompanying this paper. However, if any additional extensions of time are necessary for the consideration of this paper, such extensions are petitioned under 37 CFR § 1.136(a). Please apply any charges that may be due for extensions of time or for net addition of claims to our Deposit Account No. 50-0426.

Respectfully submitted,

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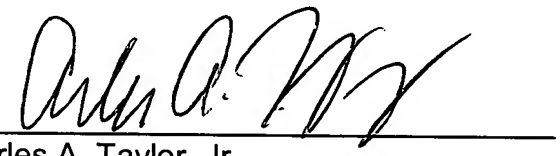
Date: May 30, 2007 By: 

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